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NOTE

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A case of pulmonary toxoplasmosis resembling multiple lung metastases of nasal lymphoma in a cat receiving chemotherapy

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ABSTRACT. An 11-year-old cat presented with nasal discharge and lacrimation and was diagnosed with nasal lymphoma. Although the cat showed favorable progression after undergoing chemotherapy, CT imaging demonstrated enlarged pulmonary nodules caused by *Toxoplasma gondii*. Following the cessation of chemotherapy, the cat was prescribed clindamycin hydrochloride for toxoplasmosis treatment; however, the cat developed kidney lymphoma and died. No *T. gondii* organisms were observed in the whole body necropsy specimens. It is known that immunocompromised human patients, including those who undergo chemotherapy, are considered at risk for toxoplasmosis. However, the risk of developing toxoplasmosis in cats undergoing chemotherapy is currently unknown. Findings from this case report suggest that cats with chemotherapy-resistant pulmonary masses might have a *T. gondii* infection rather than metastatic disease.

KEY WORDS: chemotherapy, feline, infectious disease, lymphoma, *Toxoplasma gondii* genotype III

Toxoplasmosis is one of the most common opportunistic infections among patients infected with human immunodeficiency virus (HIV) and those receiving allografts [6, 28]. Recently, it has been reported that there is an increased risk of toxoplasmosis in human patients with cancer who are undergoing chemotherapy [5, 21]. Toxoplasmosis in patients with cancer has most commonly been reported in association with hematological tumors [21], and the case of a patient with toxoplasmosis infection resembling melanoma metastasis has recently been reported [13]. Additionally, toxoplasmosis in cats, which are known to be the definitive host for *T. gondii*, has most commonly been described in association with immunocompetent kittens and immunodeficient FIV-positive adult cats [8, 17]. Furthermore, immunocompetent adult cats that were immunosuppressed by administration of glucocorticoids [8, 17] or other immunosuppressive drugs, such as cyclosporine for the treatment of immune-mediated hemolytic anemia (IMHA) or eosinophilic granuloma, or after renal transplantation, were also reported to be at increased risk for developing toxoplasmosis [2, 18]. However, toxoplasmosis due to chemotherapy has not been reported in veterinary medicine. In this case report, we describe a cat undergoing treatment with chemotherapy for nasal lymphoma, who presented with pulmonary toxoplasmosis that resembled multiple lung metastases.

An 11-year-old, male, neutered, domestic shorthair cat weighing 4.2 kg presented to the Animal Medical Center at Gifu University with a complaint of a 6-month history of right-sided nasal discharge and lacrimation that did not respond to antibiotic

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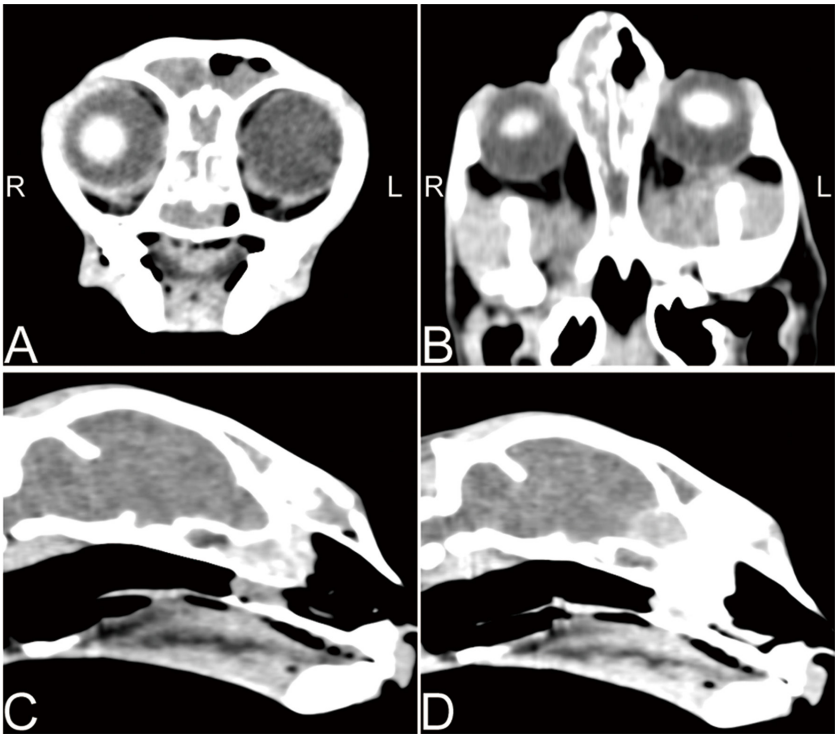

Fig. 1. Multiplanar reconstructed computed tomography images of the head of an 11-year-old male cat. Non-contrast-enhanced images in the transverse, dorsal, and sagittal plane at the level of the orbit and olfactory bulb of the brain. Soft tissue attenuating mass in the right nasal cavity was detected. The mass had also infiltrated into the right and left frontal sinus (A−C). A strongly contrast-enhanced mass had invaded into the olfactory bulb, with erosion of the cribriform plate (D).

treatment. Upon admission, the cat was febrile (40.2°C) and stuporous due to more than 10 episodes of tonic-clonic seizures, each lasting 2−3 min. Laboratory results of the complete blood count (CBC) and serum biochemical analysis, including BUN (20.9 mg/d*l*, reference interval [RI]: 17.6−32.8 mg/d*l*) and creatinine (1.1 mg/d*l*, RI: 0.8−1.8 mg/d*l*) levels, were initially within the reference limits, with the exception of total protein levels (8.6 g/d*l*, RI: 5.7−7.8 mg/d*l*). Serological tests for feline immunodeficiency virus (FIV) antibodies and feline leukemia virus antigen were negative. Computed tomography (CT) imaging (Fig. 1) revealed that an intranasal tumor had invaded the olfactory bulb; intranasal lymphoma was diagnosed after a full examination and histological analysis. The cat then underwent chemoradiotherapy with a (CHOP)-based protocol that included cyclophosphamide, doxorubicin, vincristine, and prednisone, in conjunction with hypofractionated radiation therapy for the intranasal lesion (total radiation dose: 41.3 Gy). The cat showed clinical improvement with increased appetite, and no seizures were observed after commencing treatment. On day 39 from the initial visit, the owner of the cat claimed that the cat's appetite had again decreased, and CT imaging was repeated to reevaluate the treatment response. A head CT scan revealed intranasal tumor regression, although pulmonary nodules were observed by chest CT (Fig. 2A); twelve millet grain-sized nodules were found in the right pulmonary lobes and three were found in the left. Larger nodules were detected in the right cranial lobe (14.7 mm in diameter), middle lobe (2.9 mm), and caudal lobe (3.9 mm), and a 4.8-mm nodule was detected in the left caudal lobe. On day 53, a chest CT scan revealed that the nodules had enlarged to 15.7, 4.9, and 9.0 mm in the right pulmonary lobe, respectively, and to 12.6 mm in the left caudal lobe. The millet grain-sized nodules had also increased in number; there were now 20 nodules spread throughout the right pulmonary lobes and eight nodules throughout the left lobes (Fig. 2B). At this time, the cat also began to demonstrate tachypnea.

Cytological analysis of the CT-guided needle aspirate obtained from the lung nodules revealed granulomatous inflammation caused by protozoal organisms (Fig. 3). Protozoal organisms were detected in the extracellular and intracellular space of macrophages using Hemacolor® rapid blood smear stain (Merck KGaA, Darmstadt, Germany). Large, round pseudocysts containing numerous organisms were observed within the macrophages. The organisms within the pseudocysts were oval to crescent-shaped, with a nucleus located in the broader end. The extracellular organisms were crescent-shaped, with a lightly basophilic cytoplasm, and a central metachromatic nucleus, which resembled *Toxoplasma gondii* tachyzoites. A diagnosis of *T. gondii* infection was made based on cytological findings from a fine needle aspirate (FNA) of one of the lung nodules. This diagnosis was made primarily based on the size and shape of the cyst and the presence of numerous crescent-shaped extracellular organisms.

Fecal examination was performed, but no *T. gondii* oocysts were shed in the cat feces. Subsequently, the serum of the cat was subjected to a latex agglutination test, and the FNA samples were subjected to molecular analysis using polymerase chain reaction (PCR), *in vitro* parasite culture, and genotyping.

A latex agglutination test (Toxo Test-MT) was performed following the procedures recommended by the manufacturer (Eiken

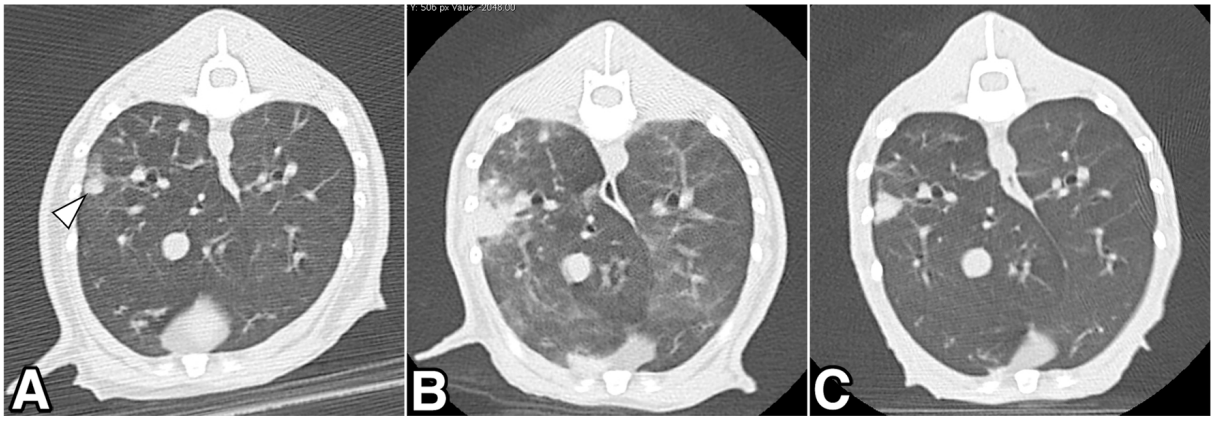

Fig. 2. Series of multiplanar reconstructed transverse computed tomography images of the thorax (pulmonary window setting) in an 11-year-old male cat. (A) A 0.6 × 0.46 × 0.72-cm irregular-shaped soft tissue nodule (arrowhead) was present in the right caudal lung lobe on day 32. (B) The nodule was enlarged (1.1 × 1.1 × 1.4 cm) with consolidation abutting the pleura. Furthermore, the nodule was poorly marginated and demonstrated a feeding vessel and a peripheral wedge-shaped. The CT values of the nodule were ranged 22-125 HU (Hounsfield Unit). The number and size of the lesions were also increased. Furthermore, a diffuse ground-glass appearance was noted on day 53. (C) The mass had shrunk (0.6 × 0.5 × 0.6 cm) and the ground-glass appearance had diminished by day 74, after 4 weeks of clindamycin hydrochloride administration. R=Right side of cat. L=Left side of cat.

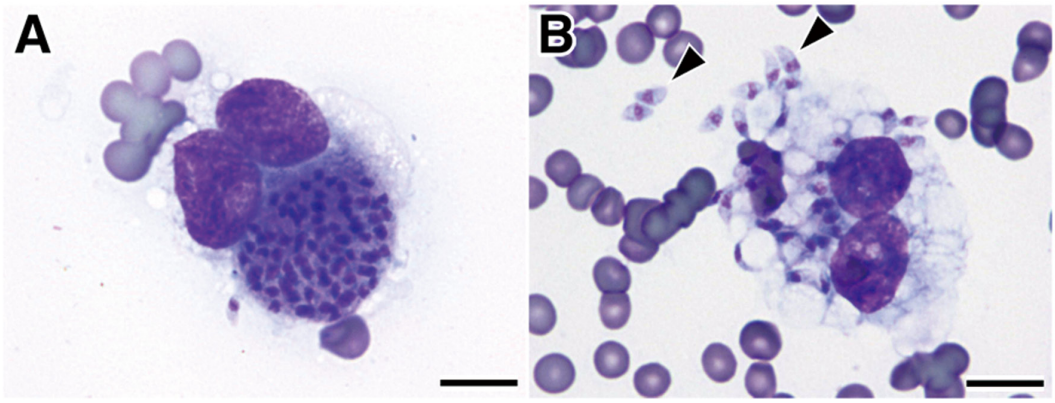

Fig. 3. Fine-needle aspirates of the pulmonary mass in an 11-year-old male cat. A cyst that contained bradyzoites can be seen within the macrophage (A). Protozoal organisms are also visible extracellularly, as 1- to 4-μm, crescent-shaped bodies with lightly basophilic cytoplasm and a central metachromatic nucleus (B), resembling *Toxoplasma gondii* tachyzoites (arrowheads). Hemacolor stain; bar=10 μm.

Chemical Co., Ltd., Tokyo, Japan). The test was positive for samples serially diluted from 1:16 to 1:256, indicating high antibody levels in the serum of the cat (positive reaction ≥64). After the cat of this case was diagnosed with toxoplasmosis, similar testing was performed on the serum of the other cats who lived together in the same home with the two families. All the cats, except for the mother of the cat in the present case, had been housed indoors all of their lives. The test was seropositive (positive reaction detected in samples serially diluted at 1:64 and 1:256, respectively) in two of the 11 cats. Seropositive results were obtained from one littermate of the cat of the present study and one cat from the other family.

For PCR analysis, DNA was extracted from the CT-guided FNA sample using the EZNA Tissue DNA Kit (Omega Bio-Tek, Norcross, GA, U.S.A.), and following the manufacturer's instructions. After extraction, PCR that targeted *T. gondii*-specific B1 gene was performed, as described by Zöller *et al*. [29]. DNA from the *T. gondii* PLK strain was used as a positive control, and distilled water was used as a negative control. The PCR amplification product was detected as expected, and the negative controls remained free of amplified products (Fig. 4).

To isolate the protozoa, Vero cells were inoculated with the aspiration sample and were cultured in RPMI-1640 medium containing 7.5% fetal calf serum; the cells were incubated at 37°C and under 5% CO_2. The cell culture medium was exchanged once a week until parasites were visually confirmed by phase-contrast microscopy. Genotyping was performed on DNA extracted from the *in vitro* parasite culture as described by Su *et al*. [25]. Results revealed a type III haplotype, as estimated by RAxML version 8.5, using the GTR + I + Γ model [26].

These cytopathological findings, together, with the results of the molecular analysis, were consistent with the histological findings of granulomatous inflammation in the lung nodules caused by a *T. gondii* genotype III strain. Immediately following diagnosis and

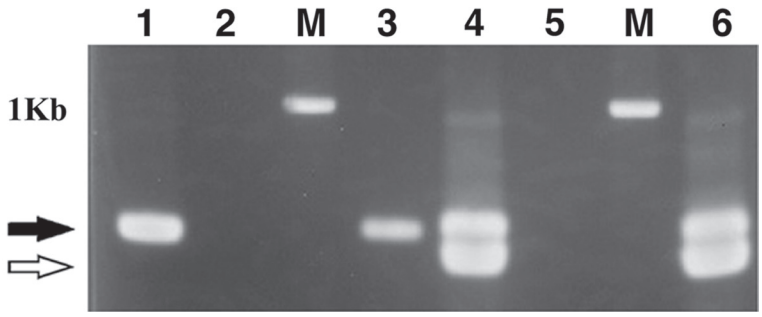

Fig. 4. Polymerase chain reaction analysis of *Toxoplasma* B1 gene in an 11-year-old male cat. A distinct band (black arrow; 469 bp) produced by the Tg1 and Tg2 primer set is visible in lane 3. A band (white arrow; 375 bp) produced by the Tgnested 1 and Tgnested 2 primer set is also seen in lane 6 (This case). Lanes 1, 4: DNA from positive control, lanes 2, 5: Negative control, M: 1 Kbp marker.

the cessation of chemotherapy for nasal lymphoma, treatment for toxoplasmosis was started, and the cat was prescribed clindamycin hydrochloride, orally (12 mg/kg, P.O., q 12 h) [17]. By the end of the following month, the cat began demonstrating partial clinical improvement, which included the return of appetite and the cessation of tachypnea; however, the cat progressively lost weight and displayed increasing weakness, despite a reduction in the size of the lung nodules on CT imaging (Fig. 2C). On day 74, the cat developed abdominal distention. Hematological analysis identified mild anemia (Hct: 27%) and serum biochemical analysis revealed several abnormalities, including increased BUN (153.1 mg/d*l*) and creatinine (4.7 mg/d*l*) levels. An abdominal ultrasonographic examination revealed markedly enlarged, hyperechoic, and irregularly shaped regions in both kidneys (right; 6.4 cm, left; 4.7 cm in diameter); using ultrasound-guided FNA, the hyperechoic areas were confirmed to be lymphoma infiltration. CHOP-based chemotherapy was re-initiated, together, with clindamycin hydrochloride; however, the cat died on day 87.

Postmortem examination was performed one day after the death of the cat, and revealed the presence of five small gray foci in the lung lobes, as well as an enlarged tan-colored kidney (Fig. 5A). Routine histopathological examination of formalin-fixed tissues revealed that CD20-positive and CD3-negative, atypical lymphoblastic lymphoid neoplastic cells had predominantly infiltrated the cortexes of both kidneys (Fig. 5B and 5C). No *T. gondii* organisms were found in any organs, including the lung nodules, kidneys, and central nervous system. This was confirmed after immunohistochemistry was performed with two types of anti-*T. gondii* antibodies, as described previously [20].

Analysis of CT-guided FNA samples obtained from the lung nodules, confirmed a diagnosis of *T. gondii* infection. Occasionally, multiple proliferating nodules in the lungs are interpreted as metastatic nodules based on the radiographic or CT appearance in patients with a primary tumor. In addition, primary and metastatic pulmonary nodules, classified as solitary, well-demarcated nodules, have been described in cats with lymphoma [7, 10, 16]. Thus, chemotherapy-resistant masses, as seen in the current case, may be indicative of toxoplasmosis. Hence, it is important to establish an accurate diagnosis of pulmonary masses/nodules. The utility of FNA cytopathology in dogs and cats has previously been reported [1, 12]. DeBerry *et al*. reported that the diagnosis obtained from cytological and histopathological specimens of the lung were the same in 82% of cases [12]. Another study also reported that the overall accuracy for diagnosis was 92% for both FNA and biopsy and that the sensitivity for diagnosing the presence of neoplasia was 91% with FNA and 80% with biopsy [1]. These reports supported the high likelihood of making a diagnosis of infectious agents in pulmonary masses.

Major complications secondary to FNA of the lung include asymptomatic pneumothorax, hemoptysis, pulmonary hemorrhage, and death when using a large (18- to 21-gauge) needle; however, the incidence of these complications is much less with the use of a smaller needle (22- to 25-gauge) [1, 12]. The needle used for FNA in our case was a 25-gauge, and no complications were observed.

It is difficult to presume how the cat in this report became infected with *T. gondii*, because the cat had B cell lymphoma. It is assumed that prednisone in the CHOP-based protocol may have induced apoptosis of B and T lymphocytes, leading to immunosuppression. Furthermore, cyclophosphamide is known to inhibit B lymphocyte preferentially [27]. Cyclophosphamide is an alkylating agent that has both potent immunosuppressive properties and antineoplastic activity, which disrupts nucleic acid function by interfering with DNA replication and RNA transcription and replication. Interestingly, Scerra *et al*. have reported that human patients who were heavily treated with chemotherapy for aggressive B cell lymphoproliferative disease were at higher risk for toxoplasmosis. It appears that chemoprophylaxis may be needed for patients who are at high risk, such as those who have B cell lymphoproliferative disorders and AIDS [21]; this may also be needed for cats with B cell lymphoma. They also described that mortality due to toxoplasmosis in hematologic malignancies was high, and that untreated toxoplasmosis was lethal [21]. Kanakry *et al*. reported that the number of naïve, potentially alloreactive conventional $CD4^+$ T cells in patients who have undergone allogeneic blood or marrow transplantation with cyclophosphamide treatment, was greatly reduced [15]. Moreover, administration of cyclophosphamide in mice resulted in increased susceptibility to infection by a non-virulent *T. gondii* strain as well as increased mortality rates [11, 23]. The cat in our study received a single cyclophosphamide treatment (200 mg/m^2, 47 mg/head) as part of the CHOP-based chemotherapy; this cyclophosphamide administration may have facilitated *T. gondii* infection and proliferation.

T. gondii genotypes have been classified into three groups depending on their pathogenicity in mice [22]. Types I and III

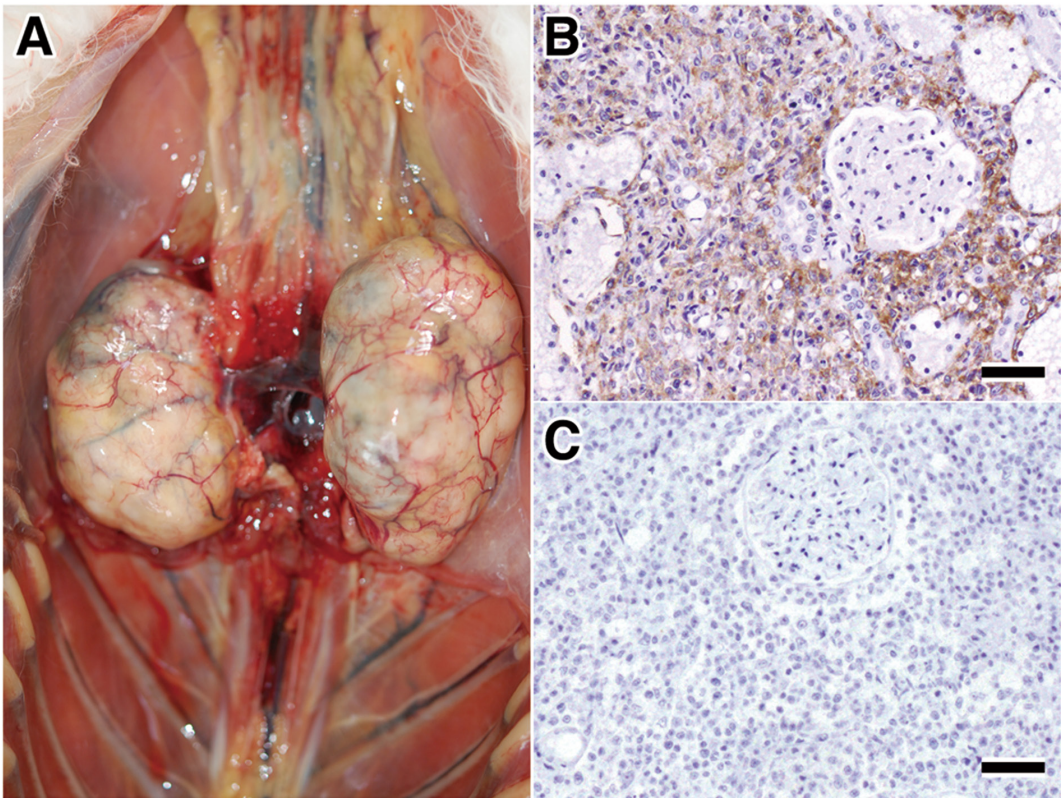

Fig. 5. Gross findings and immunohistochemistry of the kidney. (A) Photograph obtained at the necropsy of the cat showing bilateral tan-colored enlarged kidneys. (B) Immunohistochemical staining of the kidney shows strong immunoreactivity for CD20, but (C) negative immunoreactivity for CD3. Envision polymer methods were applied and DAB was used for visualization. Hematoxylin counter stain; bar=50 μm.

parasites displayed different virulence patterns in mice, with type I being highly virulent and exhibiting a 100% lethal dose (LD_{100}) profile in mice upon injection with 100 parasites. In contrast, type II exhibited low virulence ($LD_{50}>10^3$) and type III was avirulent ($LD_{50}>10^5$) [22]. Little is known about the differences in infection and outcomes between *T. gondii* genotypes in humans and cats [3, 9]. However, several studies have reported that toxoplasmosis caused by type II strains dominate in human and feline [3, 9, 14, 24]. In humans and felines diagnosed with clinical toxoplasmosis, the percentage of those infected with type III strains is reported to be 6.8–20% and 0%, respectively [3, 4, 9, 14].

The cat in this report died of lymphoma, as demonstrated by postmortem histopathological examination, although *T. gondii* infection may have influenced the risk of mortality. Chemotherapy may not be suspended when pulmonary toxoplasmosis is diagnosed, as it may allow long-term remission of the lymphoma.

A latex agglutination assay was applied to detect *T. gondii* antibodies in the cat serum in our study; thus, it is difficult to distinguish whether the cat in our case suffered from a primary or reactivated infection. However, three of the 12 cats, including this case, were positive for *T. gondii* antibodies, which may be a higher positive rate than the reported seroprevalence in Tokyo, Japan [19]. This fact suggests that toxoplasmosis may be prevalent within the household. This indicated that the cat might have suffered reactivation of a latent infection acquired before the onset of lymphoma, which may have been vertically transmitted by nursing or ingestion of oocysts during kittenhood. The fact that no oocysts were detected in the feces also supports our theory that this was a reactivation of a latent infection; in general, cats with *T. gondii* shed oocysts 1−2 weeks after infection.

The anti-*T. gondii* IgG seroprevalence was significantly higher in cancer patients (35.56%) than in non-cancer controls (17.44%), and the highest *T. gondii* seroprevalence (60.94%) among cancer patients was found in lung cancer patients [6]. According to the findings in the case reported here and in the report by Cong *et al.* [6], immunocompetent cats with cancer, which are undergoing chemotherapy, may be predisposed to *T. gondii* infection just as in human patients.

Specific guidelines for toxoplasmosis treatment in human patients with hematological malignancy are not fully formulated. Moreover, a useful strategy for treating toxoplasmosis in cats undergoing chemotherapy has not yet been determined. Cats undergoing chemotherapy may require prophylactic administration of antibiotics, as do AIDS patients.

The present case suggests that veterinary clinicians should be aware of pulmonary toxoplasmosis to avoid the misdiagnosis of lung metastasis of the primary tumor. Toxoplasmosis should be included as a differential diagnosis when considering chemotherapy-resistant masses of the lung in tumor-bearing cats; this is imperative as toxoplasmosis may develop into clinically severe disease.

CONFLICTS OF INTEREST. The authors declare that there were no conflicts of interest.

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